11:00

407-3 Glutathione Peroxidase Prevents the Inactivation of Nitric Oxide and Restores the Inhibition of Platelet Function by S-Nitrosothiols

Jane E. Freedman, Balz Frei, George N. Welch, Joseph Loscalzo. *Boston University School of Medicine, Boston, MA*

The interaction of nitric oxide (NO) with reactive oxygen species in the vasculature can inactivate NO leading to potentially adverse vascular consequences. Glutathione peroxidases (GSH-Px), a family of antioxidant enzymes present at reduced concentrations in plasma and platelets of patients with coronary artery disease, catalyze the reduction of hydrogen peroxide and lipid hydroperoxides (LOOH) by glutathione. Given the role of LOOH in platelet eicosanoid metabolism and their presence in atherosclerotic plaque, we investigated the effect of GSH-Px on the inhibition of platelet function by the naturally occurring NO donor, S-nitroso-glutathione (SNO-Glu). Subthreshold inhibitory concentrations of SNO-Glu were added to platelet-rich plasma, and aggregation was induced by arachidonate. The addition of GSH-Px (0.2–20 U/ml) to this system led to a dose-dependent inhibition of platelet aggregation with an IC_{50} = 0.6 U/ml GSH-Px ($p < 0.05$ by ANOVA). Superoxide dismutase (0.1–200 U/ml), catalase (0.1–200 U/ml), or GSH-Px without SNO-Glu did not alter platelet aggregation responses. The addi ion of GSH-Px to a subthreshold inhibitory concentration of sodium nitroprusside also did not affect platelet aggregation responses. LOOH increased platelet aggregation in the presence of SNO-Glu, an effect reversed by GSH-Px. Levels of cGMP were measured after platelets were incubated with SNO-Glu, exogenous LOOH, and GSH-Px. SNO-Glu alone increased cGMP levels, and this effect was attenuated by LOOH but restored by the addition of GSH-Px. GSH-Px activity was equivalent with either SNO-Glu or glutathione as cosubstrate. Incubation of SNO-Glu with GSH-Px led to a 48.5% decrease in the concentration of SNO-Glu as determined by HPLC-electrochemical detection. Incubation of SNO-Glu with albumin in the presence of GSH-Px led to increased formation of S-nitroso-albumin, a prevalent reservoir of EDRF in plasma. These results show that GSH-Px, at physiologically relevant concentrations, has a potent effect on NO-induced inhibition of platelet aggregation and that this enzyme may have two functions: (i) metabolism of LOOH, thereby preventing its inactivation of NO; and (ii) metabolism of SNO-Glu, thereby liberating NO and/or supporting further transnitrosation reactions. These findings suggest that GSH-Px, in addition to its antioxidant functions, regulates the availability of NO in the vasculature and possibly alters platelet-dependent thrombotic events.

11:15

407-4 Estradiol-17β Attenuates Directed Migration of Vascular Smooth Muscle Cells

Frank D. Kolodgie, Anup Jacob, Renu Virmani, Patricia S. Wilson, Greg C. Carlson, Andrew Farb. *Armed Forces Institute of Pathology, Washington, DC*

Intimal proliferation and migration of smooth muscle cells are important components of atherosclerotic progression. Experimental studies suggest an antiatherogenic property of 17β-estradiol (βE_2) independent of its effects on serum lipids and lipoproteins. Although the beneficial effect afforded by βE_2 is thought to be mediated in part by its antiproliferative properties, its action on smooth muscle cell migration is unknown. To explore this relationship, female rat aortic smooth muscle cells (RASMC) were grown in hormone-free medium and the effect of various concentrations of βE_2 on directed cellular migration was measured *in-vitro* using a microwell Boyden chamber apparatus. Immunocytochemistry demonstrated positive staining for estrogen receptors on RASMC. Migration of RASMC to the known chemoattractants platelet-derived growth factor (PDGF-BB), insulin-like growth factor-1, and fibronectin (all at maximal doses for migratory activity) was attenuated by βE_2 (0.1 to 10 ng/ml) in a concentration-dependent manner relative to control cells treated with vehicle (0.01% ethanol). This effect was insensitive to pretreatment with indomethacin and stereospecific because the enantiomer 17α-estradiol had no response. Like βE_2, the synthetic estrogen diethylstilbesterol attenuated directed RASMC chemotaxis whereas testosterone was ineffective. Further studies showed that βE_2-mediated suppression of migration was inhibited by both the antiestrogen ICI 164,384 and the gene transcription inhibitor actinomycin D. Similarly, both staurosporine and the more selective protein kinase C inhibitor GF 109203X neutralized the inhibitory effects of βE_2. [^{3}H]Thymidine uptake in PDGF-BB stimulated RASMC was not significantly affected by βE_2 in concentrations up to 100 ng/ml thus, providing further *in vitro* evidence of the importance of the effects of βE_2 on smooth muscle cell migration. These are the first results demonstrating a reduction in directed smooth muscle cell chemotaxis by βE_2. The mechanism of this βE_2-mediated response appears to involve gene transcription and is sensitive to modulation by protein kinase C.

11:30

407-5 Expression of a Mutation Causing Hypertrophic Cardiomyopathy Disrupts Sarcomere Assembly in Adult Feline Cardiac Myocytes

Ali J. Marian, Qun-Tao Yu, Douglas L. Mann, Frank L. Graham [1], Robert Roberts. *Baylor College of Medicine, Houston, Texas, USA; [1] McMaster University, Hamilton, Ontario, Canada*

Mutations in the β myosin heavy chain (βMHC) gene cause hypertrophic cardiomyopathy (HCM), a disease characterized by sudden death, cardiac hypertrophy and sarcomere disarray. To determine the primary defect, we sought to express a mutant βMHC gene in adult feline cardiac myocytes. The feline model was selected since HCM is a common disease in cats with a phenotype identical to that of man and the cardiac myosin is βMHC as in man in contrast to αMHC in the mouse. A full length human βMHC cDNA was cloned for the first time and an HCM causing mutation ($Arg^{403}Gln$) was induced by site-directed mutagenesis. The adenoviral vector was selected for its high efficiency of gene transfer and reconstructed to accommodate the 7 kb βMHC cDNA and promoter. The normal and mutant βMHC cDNAs were cloned into a shuttle vector (ΔE1sp1B) downstream to a cytomegalovirus promoter and the 7 kb fragment was incorporated through homologous recombination during co-transfection of pΔE1sp1B/CMV/βMHC and pBHG10 in 293 host cells. Adult feline cardiac myocytes were transfected with the recombinant viruses and examined at 48 and 120 hours after transfection. Expression of the βMHC into mRNA was confirmed by RT-PCR. Net myosin synthesis, determined by ^{3}H phenylalanine incorporation (48 hr), was greater in myocytes with normal or mutant βMHC constructs than control myocytes or those infected with vector alone ($p < 0.05$). Electron microscopy showed normal sarcomere assembly at 48 hours in >90% of myocytes, however, at 120 hours sarcomere disruption was evident in >50% of myocytes infected with mutant βMHC but remained intact in control myocytes and those infected with normal βMHC or vector alone ($p = 0.03$). In this study, we successfully incorporated a 7 kb βMHC/promoter construct into an adenoviral vector and showed its expression in adult feline cardiac myocytes. The mutant βMHC induced sarcomere and filament disarray similar to the lesion seen in man. This would indicate sarcomere disarray is the primary defect in HCM and the hypertrophy is compensatory.

11:45

407-6 Intraarterial Beta Irradiation Prevents Neointimal Hyperplasia in a Hypercholesterolemic Rabbit Restenosis Model

Vitali Verin, Youri Popowski, Philip Urban, Jacques Belenger, Mireille Redard, Manuel Costa, Marie-Claude Widmer, Michael Schwager [1], John Kurtz, Wilhelm Rutishauser. *Cardiology Center and Division of Radiation Oncology, University Hospital, Geneva, Switzerland; [1] Schneider, Europe, AG*

Intraarterial gamma irradiation reduces restenosis following balloon angioplasty. Beta irradiation has the advantages of a markedly steeper dose decrease in tissue and less radioprotection problems, permitting its use in the setting of an ordinary catheterization laboratory. Flexible yttrium coils (diameter 0.016", length 24 mm) were activated in a nuclear reactor. Use of a segmented balloon consisting of four interconnected compartments (*Schneider, Europe, AG) allowed for intraarterial centering of the 90Y source and homogeneous intramural dose delivery. Under fluoroscopic guidance, one carotid and one iliac artery of 21 hypercholesterolemic rabbits were deendothelialized and then simultaneously dilated and irradiated. Four dose schedules were studied: 1) control (dilated, non irradiated); 2) 6 Gray (Gy); 3) 12 Gy and 4) 18 Gy. Arterial specimens were histologicaly evaluated at 8 days and at 6 weeks. A minimum of 5 arteries were studied in each group at each study end-point. There was a significant decrease ($p < 0.001$–0.05) in